# Effect of adding functional lactic acid bacteria at ensiling on the fermentation quality and nutritive value of oat silage

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## Introduction

Livestock are exposed to stressors from the environment such as temperature, humidity and intensive feeding. Strong stress can cause animals to perform an atypical behavior and/or result in lower productivity, and livestock weanlings, which do not have as strong a body as the adult livestock, are more severely affected by stress. Functional silage that increases the stress tolerance of livestock may thus be beneficial. Some lactic acid bacteria (LAB) in human food were found to have a probiotic effect to ease stress, but it has not been established whether these LAB can affect the qualities of silage fermentation. Here we used a small-scale system of silage fermentation to examine the effects of adding functional LAB at ensiling on the fermentation quality and nutritive value of oat silage.

### Methods

Oat (Avena sativa L.) was seeded on October 28, 2011 in Hakusan, Ishikawa, Japan (36°N, 136°E) and harvested on June 5, 2012. The harvested oat was cut to 22 mm with a hay chopper. The silage was prepared using a small-scale system for silage fermentation. Functional LAB named ANP7-1, ANP7-4 and ANP7-6 were used (Table 1). Suspensions of ANP7-1, ANP7-4, and ANP7-6 were prepared by the plate culture method. The inoculum size of the LAB was 2 mL of suspension/kg fresh matter (FM). A commercial LAB additive, Chikuso-1 (Snow Brand Seed Co. Hokkaido, Japan) was used as one of the control treatments, and the inoculum size was  $5 \times 10^{-3}$  g/kg FM. The five silage treatments were designed as follows: untreated control, Chikuso-1 as the positive control, ANP7-1, ANP7-4 and ANP7-6. Approximately 150-g portions of chopped Oat were packed into plastic film bags, and the bags were sealed with a vacuum sealer. The small-scale system was opened about 2 months after packing. Forty grams of the silage was mixed with 140 mL of distilled water to prepare the silage extract solution by the method of Cai (2009). The pH of the extract solution was determined with a pH meter. Organic acids and the ammonia concentration were determined with an HPLC (high-performance liquid chromatography) system and the indophenol method, respectively. Dry matter (DM) was determined by drying silage at 100°C, and total nitrogen was determined with a nitrogen and carbon analyzer. Chemical compositions were determined and total digestible nutrients (TDN) were

 Table 1. Characteristics of the functional lactic acid bacteria used.

	Name	Туре	Fermented type	Effect
ANP 7-1	L. plantarum	bacillus	homo	Immunity activation
ANP 7-4	L. paracasei	bacillus	homo	Allergy control
ANP 7-6	L. brevis	bacillus	hetero	GABA production, high blood- pressure control

calculated by the method of Abe (1988). The V-Score was calculated by the method of Cai (2009). The data were subjected to an analysis of variance, and the effect of the silage treatments was determined. The Tukey method was used to compare the means among the treatments when the effect was significant. IBM SPSS software for PCs was used for the statistical analysis.

### Results

The fermentation qualities of the oat silage are shown in Table 2. The pH was significantly lower in the ANP7-1, ANP7-4 and ANP7-6 treatments than in the no-addition and Chikuso-1 treatments, and the pH in the ANP7-4 treatment was significantly lower than that in the ANP7-1 and ANP7-6 treatments. Lactic acid was significantly higher in the ANP7-4 and ANP7-6 treatments than in the no-addition and Chikuso-1 treatments. Acetic acid, propionic acid and butyric acid were significantly lower in the Chikuso-1, ANP7-1, ANP7-4 and ANP7-6 treatments than in the no-addition treatment. Iso-butyric acid, isovaleric acid and n-valeric acid were not detected in all treatments. Ammonia was significantly lower in the ANP7-1, ANP7-4 and ANP7-6 treatments than in the no-addition and Chikuso-1 treatments. The V-score was significantly higher in the Chikuso-1, ANP7-1, ANP7-4 and ANP7-6 treatments than in the no-addition treatment.

The chemical fractions with higher digestibility such as organic cellular content and nitrogen-free extracts were significantly higher and the lower digestibility fraction of Ob was significantly lower in the Chikuso-1, ANP7-1, ANP7-6 treatments than in the no-addition treatment. Crude protein was significantly lower in ANP7-1 and

Items	Silage treatments					
	No-addition	Chikuso-1	ANP7-1	ANP7-4	ANP7-6	
Moisture	75.49 a	70.16 c	75.43 a	72.63 b	71.04 bc	
pH	4.69 a	3.91 b	3.78 c	3.70 d	3.77 c	
Organic acids						
Lactic acid	0.37 d	1.52 cd	1.63 bc	1.79 a	1.71 ab	
Acetic acid	0.36 a	0.13 b	0.12 b	0.08 b	0.12 b	
Propionic acid	0.03 a	0.01 b	0.01 b	0.01 b	0.02 ab	
Butyric acid	0.47 a	0.04 b	0.01 b	0.01 b	0.00 b	
Ammonia (mg/100g)	32.74 a	21.97 b	13.09 c	9.98 c	15.40 c	
Total nitrogen (g/100g)	1.00a	1.02 a	0.90 b	0.93 b	1.00 a	
V-Score	61.48 b	96.5 a	99.18 a	99.33 a	99.86 a	

Table 2. Effect of addition of functional lactic acid bacteria on the fermentation quality of oat silage.

Means with different letters in the same row of each treatment differ significantly (P<0.05).

#### Table 3. Effect of addition of functional lactic acid bacteria on the chemical composition and nutrition value of oat silage

Items	Silage treatments						
	No addition	Chikuso-1	ANP7-1	ANP7-4	ANP7-6		
OCC	37.35 b	42.49 a	38.44 b	40.59 ab	42.74 a		
Crude protein	6.26 a	6.38 a	5.60 b	5.79 b	6.28 a		
Crude fat	3.83 a	3.87 a	3.27 b	3.66 ab	3.90 a		
NCWFE	28.92 c	33.93 ab	31.18 bc	32.76 ab	34.23 a		
OCW	54.52 a	50.38 b	54.21 a	52.22 ab	50.27 b		
Oa	7.12	8.45	8.51	8.11	7.43		
Ob	47.40 a	41.93 c	45.71 ab	44.11 bc	42.84 c		
TDN	59.28 c	63.17 a	61.02 b	61.99 ab	62.86 a		

OCC: organic cellular content, NCWFE: nitrogen free extracts, OCW: organic cell wall, Oa: higher digestible cell wall, Ob: lower digestible cell wall, TDN. TDN: total digestible nutrients.  $TDN = 1.111 \times (OCC + Oa) + 0.605 \times Ob - 18.8$  (Abe 1988). Means with different letters in the same row of each treatment differ significantly (*P*<0.05).

ANP7-4 than in the no-addition, Chikuso-1, and ANP7-6 treatments. TDN was significantly higher in Chikuso-1, ANP7-4 and ANP7-6 than in the no-addition treatment.

## Conclusion

The functional LAB of ANP7-1, ANP7-4 and ANP7-6 were found to improve the oat silage fermentation quality as effectively as the commercial LAB additive, Chikuso-1. ANP7-1 and ANP7-6 were shown to enhance the nutritive value of oat silage as well as Chikuso-1 did. These results indicated the potential use of ANP7-1, ANP7-4 and ANP7-6 as silage additive.

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